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DEMONSTRATION OF MUSCLE INJURY IN EXPERIMENTAL BULLET WOUNDS [R]

by

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SUMMARY

Various macroscopic staining methods of demonstrating the extent of muscle injury in standard experimental thigh wounds of sheep due to military rifle bullets are described.

2:3:5 Triphenyl tetrazolium chloride (TTC) staining has been developed as a simple method for assessing the extent of muscle injury after wounding by a bullet.

It is suggested that the injury thus demonstrated is caused by the 'cavitation' effect but additional studies are required to eliminate other possible causes.

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DEMONSTRATION OF MUSCLE INJURY IN EXPERIMENTAL BULLET WOUNDS

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R. SCOTT (Lieutenant Colonel RAMC)

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INTRODUCTION

Military rifle bullets in their passage through the body at normal military ranges may cause injury to the tissues that extends beyond the immediate permanent missile track (Scott 1974). In a study of standardised experimental wounds of sheep thigh muscles produced by a hunting bullet Hopkinson and Watts (1963) found that there is a zone surrounding the missile track in which the muscle fibres stain blue with Giemsa in contrast with the pink staining of normal fibres. Further work demonstrated that this blue staining seen microscopically was associated with structural changes in muscle fibres (Hopkinson 1964).

This result was confirmed using military rifle bullets to inflict standard injuries at short range. In addition macroscopic staining methods were developed and the extent of abnormal staining of whole tissue compared with the extent of abnormal muscle fibres demonstrated by Giemsa staining technique.

MATERIALS AND METHODS

Wounds

Adult sheep of mixed breed were anaesthetised with intravenous nembutal 2 ml/5 kg body weight. Intra-muscular Rompan was administered subsequently to maintain anaesthesia during the period of the experiment in initial experiments up to 6 hours. Parallel work indicated that a standard soft tissue injury in sheep was not accompanied by any degree of apparent discomfort for the animal and recovery from anaesthetic was permitted in later experiments. The missile track in each case was posterior mid-thigh lateral to medial.

Animals were killed with humane killer at 1, 3, 5, 6, 12 and 18 hours after wounding.

Wounds were inflicted by standard military 7.62 mm and 5.56 mm ammunition fired from barrels on fixed rests 13.7, 100, 300 and 500 metres. The detailed histological

staining and macroscopic techniques were worked out on short range wounds and used to demonstrate injury caused by bullets at longer, more realistic, ranges.

Macroscopic demonstration techniques

Indian ink perfusion:- The killed animal was exsanguinated and eviscerated. The abdominal aorta and inferior vena cava were cannulated and 6 litres of normal saline at 37°C containing 0.25% sodium nitrite perfused through the hind quarters from a pressure head of 1.5 metres. At the completion of the saline perfusion 100 mls of HMSO Waterproof Indian Ink was run in through the arterial cannula. When the ink appeared in the venous cannula this was clamped and the pressure head raised before the arterial cannula was clamped. Three hours later the clamps were removed, the hind limbs skinned and deep frozen. After 48 hrs whole limb sections 1 cm thick were cut on a band saw, mounted, measured and photographed.

Macroscopic staining reactions:- Two methods of limb preparation were used. In the first the animal was eviscerated after death and the hind quarter removed and skinned, left at room temperature up to 1 hour and then deep frozen for 48 hours. The frozen limb was sliced into 1 cm whole sections by a band saw, allowed to defrost at room temperature and washed in normal saline.

In the second method the animal after death was eviscerated, skinned and hung in a cool room (about 15°C) for 24 hours. The hind quarter was amputated, the femur removed without disturbing the wound track and surrounding muscles and the posterior thigh muscle mass sliced with a bacon slicer into 0.5 cm thick slices, which was the minimum thickness obtainable, while keeping the section intact.

Indicator stain:- The whole section slice was immersed in a freshly prepared alcoholic solution of saturated Bromothymol blue (4 mgms % BDH stain in 70% alcohol) for 15 minutes at room temperature. It was then removed from the stain, blotted dry and photographed with Ektachrome or Kodachrome II film.

TTC:- Sections were incubated in a prewarmed solution of 1% TTC (2:3:5 Triphenyl tetrazolium chloride) in mixed acid-phosphate buffer pH 8.5 for 15-30 minutes, being removed when maximum differentiation achieved, and transferred to 10% formol saline solution to preserve and enhance the colour changes, which were recorded on colour film.

Nitro BT:- Sections were incubated in 75 mls of NBT solution [0.5 mgm/ml. 2,2^1-di-p-nitrophenyl 5,5^1-diphenyl 3,3^1 (3,3^1-dimethoxy 4,4^1-biphenylene) ditetrazolium chloride in Sorensens phosphate buffer 0.1 M at pH 7.4] at 37°C for 30 minutes, removed, washed with normal saline then placed in 10% formol saline and photographed.

RESULTS

In the first series of experiments, wounds from 35 subjects were examined by one or more of the methods described above; this investigation was only part of a series of bullet wound studies. All limbs in this series were deep frozen before staining.

Indian ink perfusion:- Six perfusions were completed. In each instance a wound had been inflicted at short range, by a 7.62 mm bullet. In three instances there was an area surrounding the missile track, which did not take up the Indian ink and remained red contrasting with the deep black of the surrounding perfused muscle. There was considerably variation in extent of this area at different levels in the limb and in different limbs.

Indicator stain:- Eight wounds were examined, each giving 7-9 one cm slices. The slices showing the maximum area of abnormal staining were compared with each other. In every instance there was an area round the missile track which stained green and contrasted with the yellow staining of the "normal" muscle.

The green area surrounding the missile track increased as the time from wounding increased up to 6 hours.

Nitro BT stain:- Eight wounds were examined. Surrounding the missile track in each case there was an area which did not react with the formazan dye to produce the dark blue staining of normal muscle. This area increased up to 6 hours from wounding.

TTC stain:- Eight wounds were examined. Surrounding the missile track in each case there was an area which did not react to produce the pink formazan dye which stained normal muscle. This area increased up to 6 hrs from wounding. Five wound preparations were left unstained.

This series of investigations indicated that there was a volume of muscle surrounding the missile track after wounding which did not perfuse normally, had a slightly raised pH, and did not reduce tetrazolium dyes. This volume of abnormally staining muscle increased up to 6 hours after wounding. Six hours was the longest period of observation in this series.

In a further series of 8 wound studies thin slices were cut using the second method of preparation. TTC staining only was performed. The wounds were all inflicted by the same calibre (7.62 mm) bullet at the same range (13.7 metres). Two animals were killed at 3 hours, 6 hours, 12 hours and 18 hours after wounding. The size of the abnormal area around the wound track and the differentiation of normal from abnormal increased up to 18 hours. In all cases histological section showed that the boundary of the abnormal staining reaction to TTC macroscopically was the boundary of abnormal muscle as shown after staining by Giemsa and Haemotoxylin and Eosin using Hopkinson's criteria (Hopkinson 1964).

DISCUSSION

Dimond and Rich (1967) drew attention to the severity of wounds inflicted by M-16 rifle ammunition in Vietnam. Amato, Rich, Billy, Gruber and Lawson (1974) have attempted to define the causative mechanism of injury to blood vessels by shooting the hind limbs of dogs with 16 grain spheres shot at 1000, 2000 and 3000 ft/sec. X-rays of 1 μsec duration taken during the passage of the sphere demonstrate cavitation in the tissues at the higher velocities. In further experiments an M16 rifle bullet was shown to damage blood vessels outside it's immediate track but within the cavity (Amato and Rich 1972).

It has been suggested that cavitation damage is only significant when the impact velocity of the missile is above 700-800 metres/sec (Lewis et al 1975, Clemedson et al 1973) and that the rapidly expanding temporary cavity produces blunt contusion effects on small blood vessels giving rise to loss of vascular tone in macroscopically non-affected tissues. In the wound channel, angiograms show disruption, extravasation of contrast medium, major changes of calibre and more pronounced dislocation of blood vessels (Lewis, Rybeck, Sandegard, Seeman and Zachrisson 1974).

Hopkinson and Watts (1963) studying experimental sheep-thigh wounds caused by hunting bullets showed that the extent of the area in which capillaries are unperfused with Indian ink increased after wounding, reaching a maximum in the middle

of the wound at about 6 hours from wounding. Up to three days from injury, this zone was more extensive than the zone of hyalinized fibres with pyknotic nuclei seen on microscopy after Giemsa staining.

Hopkinson (1963) suggests that there are three areas in the cross-section of a penetrating missile wound of muscle. The area of complete tissue disruption due to laceration and crushing is surrounded by an area of abnormally staining muscle, staining reactions of which indicate a physical change in the muscle fibres. Surrounding this area is a further area in which there is lack of filling of small blood vessels and extravasation of blood. The wound channel decreases during healing, the "necrotic area" increases at first to meet the margin of the "ischaemic area" at about 3 days after injury and they then both decrease together during the repair process.

Experimental work suggests that cavitation causes the injury round the permanent wound track associated with the passage of a military bullet. Cavitation occurred in tissue simulants at impact velocities of military bullets below 800 metres per second and macroscopic tissue damage surrounded the permanent track of military bullet injuries in experimental animals at long range, where the impact velocity of the bullet was below this figure (Scott 1974).

TTC staining is a rapid simple method of assessing the extent of muscle damage in a standard experimental thigh wound due to a military bullet.

Comparisons of the extent of such wounding due to different bullets, should be made at similar ranges, and at the same time after wounding.

Further work is required to demonstrate whether mechanisms other than cavitation injury of small blood vessels is responsible for tissue damage, and to elucidate the nature of the cellular damage demonstrated by TTC staining.

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